Bridging bench and bedside to explore the role of Indian polyvalent antivenom in treating snakebite in Sri Lanka

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Introduction

Snakebite continues to be a significant problem in the lives of millions of people in rural, agricultural communities across south and south-east Asia, sub-Saharan Africa, and Latin America.[1] The World Health Organization (WHO) has re-designated snakebite as a neglected tropical disease due to its high incidence, severity, and clinical characteristics despite lacking epidemic potential.[2] Common acute clinical effects of snake envenoming include effects at the bite site such as local tissue necrosis and systemic effects such as venom-induced consumption coagulopathy (VICC), neuromuscular paralysis, myotoxicity, acute kidney injury and thrombotic microangiopathy.[3,4] The commonest acute systemic effect is VICC, which occurs due to the gradual consumption of clotting factors due to the action of snake procoagulant toxins. In addition to acute effects, long-term effects such as chronic musculoskeletal effects, chronic kidney disease, psychological effects, and blindness may continue to affect the lives of snakebite survivors.[5-8] Antivenom is the only specific treatment available that has become the standard practice in the treatment of snakebites over the last 120 years across the globe.[9] However, there are still significant knowledge gaps, including a poor understanding of the pathophysiology of envenoming and the lack of effective interventions, which hinder global efforts to combat snakebite.[10]

Antivenoms are polyclonal whole IgG molecules (150kDa) or their fragments, such as F(ab')2 (110kDa) or Fab' (50kDa), raised against one or several snake venoms in animals such as horses and sheep. Their primary action is to bind with venom antigens in the circulation, forming large venom-antivenom complexes. This process traps toxins within the circulation and facilitates their elimination.[9] The efficacy of an antivenom is the ability of its antibody molecules to bind with venom antigens under 'controlled conditions', and the effectiveness of an antivenom is the ability of antivenom to prevent or reverse clinical effects of envenoming in a clinically detectable manner.[9] The efficacy of antivenom against snake venom does not guarantee its clinical effectiveness. Clinical effectiveness depends on factors beyond efficacy, such as the reversibility of the pathophysiology caused by the snake toxins, as well as the pharmacokinetics and pharmacodynamics of the antibodies in the antivenom.[9,11,12]

Snakebite is a significant public health concern in Sri Lanka, with one of the highest incidence rates in the world. Community-based estimates indicate 398 snakebites, 151 envenomings, and 2.3 deaths per 100,000 population annually.[13] In the North Central Province, the incidence rates of snakebites and envenomings are approximately two and three times higher, respectively, compared to the national figures of Sri Lanka. In Sri Lanka, only five snakes are considered of the highest medical importance due to their frequent bites, which cause systemic envenoming that requires immediate medical attention. These snakes are Russell's viper *(Daboia russelii)*, Merrem's hump-nosed viper *(Hypnale hypnale)*, Indian krait *(Bungarus caeruleus)*, Common cobra *(Naja naja)*, and

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Saw-scaled viper *(Echis carinatus)*. The only available antivenom in Sri Lanka is the Indian polyvalent antivenom, which is raised and used against all the mentioned snakes except Merrem's hump-nosed pit viper. The effectiveness of Indian polyvalent antivenom in reversing the effects of envenoming by Sri Lankan snakes, such as Russell's viper, Indian krait, and common cobra, has previously been questioned.[14-16] Indian polyvalent antivenom is known for frequent, severe acute adverse reactions[17] as well as delayed reactions such as serum sickness.[5] Hence, there are serious safety concerns.

The Indian polyvalent anti-venom is produced by immunising horses with snake venoms collected from India. The observed ineffectiveness of Indian polyvalent antivenom in Sri Lanka has been attributed to the geographical differences in the venom compositions between Sri Lankan and Indian populations of snakes. This assumption, along with the high rates of acute adverse reactions including anaphylaxis[17] has provided justification for the need for a geographically specific antivenom for snake envenoming in Sri Lanka.[18,19]

Is Indian polyvalent antivenom truly ineffective for snake envenoming in Sri Lanka? Is the observed 'failure' of Indian polyvalent antivenom in Sri Lanka due to lack of efficacy? Do we adequately understand the limitations of antivenom therapy and the possible solutions? Answering these questions requires a meaningful linking of clinical studies and laboratory experimentation.

Laboratory testing of antivenom efficacy

Since snake venoms are complex cocktails of diverse toxins with diverse pharmacological properties, the net toxic effect of snake venoms, in the form of their ability to kill an organism (lethality), is tested. The widely accepted test of the lethality of snake venom is the Median Lethality Dose (LD_{50}) estimation, where the different venom doses are given to groups of laboratory rodents, such as mice, via different routes, usually intravenously. Irrespective of the cause of death, the death rates within each dose group over 48 hours are considered for LD_{50} calculation. Most importantly, the pre-clinical testing of the clinically used antivenoms, as the WHO recommends, is based on the LD_{50}. This pre-clinical test, which is the lethality prevention test or the Median Effective Dose test (ED_{50}), is the median antivenom dose that prevents 50% of the rodent deaths caused by five times the LD50 dose of the tested venom.[20] WHO guidelines mandate that antivenom manufacturers use this test to determine the efficacy of all antivenom batches used for snakebite patients worldwide.

However, alternative tests directly measure the ability of antivenom molecules to bind with the clinically important venom toxins in vitro. The in vitro venom-antivenom binding tests demonstrate the ability of antivenom molecules to directly bind with venom toxins outside a living animal or under 'ideal conditions'. The in-vitro nerve-muscle preparations, such as chick-biventer nerve-muscle preparation, specifically test the ability of the antivenoms to prevent and reverse the neuromuscular block caused by snake venoms at the neuromuscular junction. In addition, the same pre-paration is used to test the antivenom efficacy against the myotoxicity induced by the venom. The in-vitro coagulation assays test the prevention of pro-coagulant activity of venoms by the antivenom.[11,21]

Rodent lethality tests for antivenom efficacy: How relevant are they for humans?

A fundamental assumption of using rodent lethality assays in testing antivenom efficacy is that the physiological responses of rodents to snake venom adequately represent that of humans.

Many snake venoms contain neurotoxins, and two sites in the neuromuscular junction are the major targets of them: the pre-synaptic nerve terminal and post-synaptic nicotinic acetylcholine receptor (nAChR). Many snakes that cause paralysis in humans have potent pre-synaptic neurotoxins (β-neurotoxins) in their venoms. The toxins that act post-synaptically (α-neurotoxins) block the neurotransmission by high affinity, selective binding to and antagonism of the nicotinic nAChR in the motor end plate.[22,23] Based on electrophysiological studies that tested the binding of different α-neurotoxins with the nAChR with human and rat nAChR expressed on frog oocytes, it was shown that a major group of α-neurotoxins are less potent and highly reversible in human nAChR but are highly potent and poorly reversible on rat nAChR.[24] This means that, in the lethality assays, rats die due to toxins that are not clinically relevant for humans, and therefore, the ED_{50} assays may not be suitable to test the efficacy of antivenoms for human use. Similarly, rodents are found to be resistant to clinically highly important toxins, such as pro-coagulant toxins, which are responsible for VICC in humans.[25] Therefore, lethality tests such as LD_{50} and ED_{50} could be erroneous and misleading within the context of snake envenoming in humans. However, even today, the antivenoms that are clinically used in the world are tested for their efficacy using these lethality tests, despite the fact that concerns are raised against the use of these tests.[26]

Clinically relevant in-vitro tests show the efficacy of Indian polyvalent antivenoms against medically important Sri Lankan snake venoms

Using a range of tests such as in-vitro tests of venom-antivenom binding, in vitro nerve-muscle preparations and the in-vitro pro-coagulant activity assays, the efficacy of two brands of Indian polyvalent antivenom that are routinely used in Sri Lankan hospital setting, against Russell's viper *(Daboia russelii)*, Saw-scaled viper *(Echis carinatus)*, common cobra *(Naja naja)* and Indian krait *(Bungarus caeruleus)* venoms from Sri Lanka have been tested previously.[21] In contrast to the common 'belief', a reasonable efficacy of Indian polyvalent antivenoms in binding with venom antigens of all four tested venoms in vitro was shown in the above study. The Indian polyvalent antivenoms neutralised the procoagulant activity of Russell's viper and Saw-scaled viper venom from Sri Lanka, which causes VICC in humans. Another study demonstrated that the Indian polyvalent antivenom neutralises the procoagulant activity of Saw-scaled viper venom but unable to neutralise the procoagulant activity of Merrem's hump-nosed pit viper *(Hypnale hypnale)* venom from Sri Lanka.[27]

In addition, in chick-biventer nerve-muscle preparation-based experiments, the ability of Indian polyvalent antivenoms to neutralise the clinically important pre-synaptic neurotoxicity of Indian krait venom from Sri Lanka was demonstrated.[28] In another study that used the same preparation, neurotoxic activity of Indian cobra *(Naja naja)* venom from Sri Lanka, which leads to neuromuscular paralysis, was shown to be neutralised by Indian polyvalent antivenom.[29] Further, another study demonstrated that the local myotoxicity induced by Indian cobra venom from Sri Lanka could be neutralised by the Indian polyvalent antivenom.[30] Similarly, using the same in-vitro preparations, the neurotoxicity induced by Sri Lankan Russell's viper venom *(Daboia russelii)* [31] and the myotoxicity induced by the same venom[32] were shown to be neutralised by Indian polyvalent antivenom.

Therefore, the above experimental studies clearly demonstrated that Indian polyvalent antivenoms contain antibodies that can bind and neutralise the toxic effects that are clinically important for humans.

Indian polyvalent antivenom accelerates the recovery from VICC caused by Sri Lankan Russell's viper envenoming

A recent Cochrane systematic review has shown that, despite antivenoms being in use for snake envenoming for over a century, no randomised placebo-controlled studies have been conducted anywhere in the globe.[33] Even among the non-randomised comparative trials that concluded the effectiveness of antivenom for VICC, only one study was found to have included an untreated group of envenomed patients to have a meaningful comparison.[34] That study demonstrated that antivenom shortens the recovery time from VICC in Saw-scaled snake (*Echis* spp.) bite patients in North Africa.[35]

Indian polyvalent antivenom has been in use for VICC in Russell's viper envenoming in Sri Lanka without solid clinical evidence for several decades. Therefore, an observational study was conducted based on the Anuradhapura Snakebite Cohort, which compared[39] Russell's viper envenomed patients with VICC who received antivenom and five who did not receive antivenom due to falsely negative whole blood clotting test.[36] Despite the antivenom group having more severe VICC, 27/39 patients (69%) in the antivenom group had an INR < 1.5 at 48 hours post-bite compared to 0/5 patients (0%) in the no antivenom group. The fibrinogen recovered in 32/39 patients (82%) in the antivenom group compared to 1/5 patients (20%) in the no antivenom group by 48 hours. The antivenom accelerated the recovery of VICC in patients with Russell's viper envenoming, compared to no recovery in a smaller group of patients with milder VICC who did not receive antivenom. Therefore, the Indian polyvalent antivenom appeared clinically effective for VICC in Russell's viper envenoming in Sri Lanka. This effectiveness is likely due to the binding and/or neutralisation of procoagulant toxins in the circulation, allowing the liver to replace the consumed clotting factors over the next few days.

Indian polyvalent antivenom is efficacious but ineffective in reversing neuromuscular paralysis in Indian krait and Russell's viper envenoming in Sri Lanka

Using serial single-fibre electromyography (sfEMG) studies and serial measures of venom concentration using specific venom-detection ELISA, an observational study that included[33] authenticated Indian Krait bite patients was conducted as a part of the Anuradhapura Snakebite Cohort.[37] Both clinical and neurophysiological evidence suggested that the antivenom could not prevent or reverse the neuromuscular paralysis in Indian krait envenoming. After the first dose of 20 vials of Indian polyvalent antivenom, the circulating free venom was immediately undetectable in all patients, indicating the efficacious binding of the antivenom molecules with the venom antigens. However, as evident from sfEMG and the clinical picture, patients had only mild paralysis when

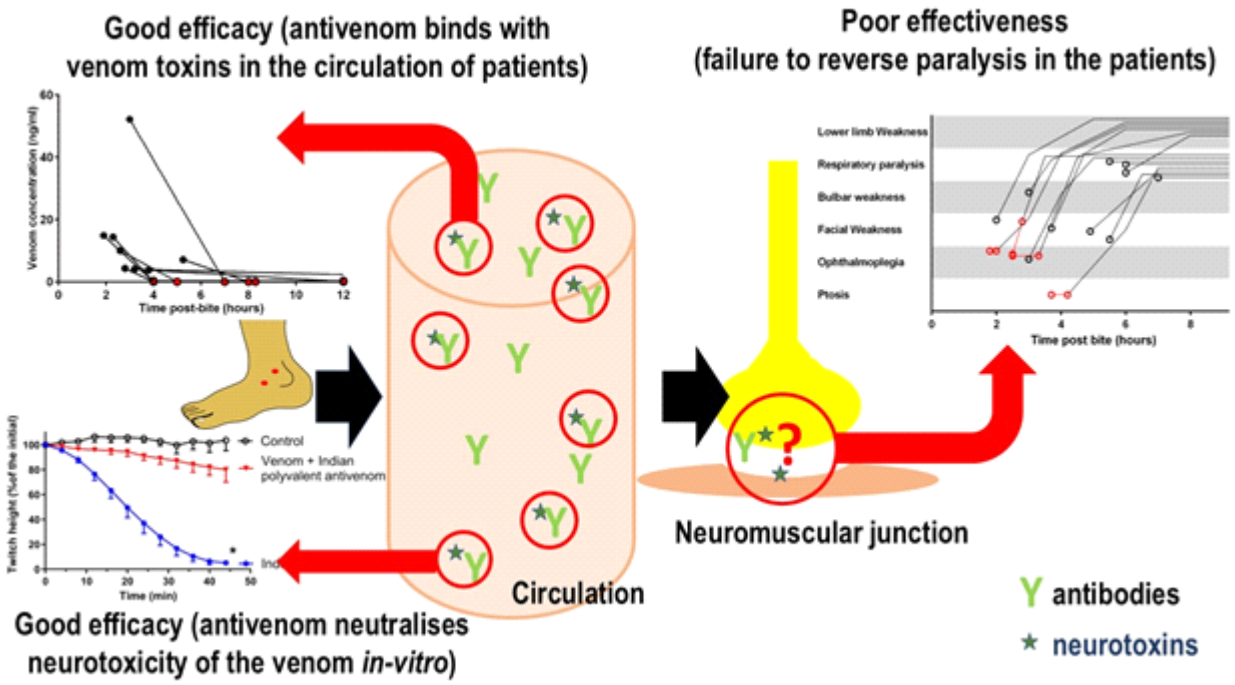

Figure 1. Lack of effectiveness of Indian Polyvalent antivenom in preventing the worsening of paralysis in Indian krait envenomed patients, despite the antivenom neutralises venom toxins in the blood and neutralising the neurotoxicity of venom in-vitro, indicating good efficacy. (Includes figures adopted from Silva et al. PLoS Negl Trop Dis 2016; 10(2):e0004368. https://doi.org/10.1371/journal.pntd.0004368 and Silva et al. Toxins 2016;8(10):302. https://doi.org/10.3390/toxins8100302, both published under Creative Commons license 4.0).

they received antivenom, but antivenom did not prevent them from progressing into life-threatening paralysis. This means the antivenom has cleared venom from the circulation, but there was no apparent response at the neuromuscular junction, suggesting that the anti-venom was efficacious but ineffective for neuromuscular dysfunction caused by Indian krait envenoming in Sri Lanka (Figure 1).

In another study that followed a similar methodology, Russell's viper envenoming led to neuromuscular paralysis limited to ptosis and ophthalmoplegia in 130 of 245 (53%) patients.[38] Unlike in common krait bite patients, the sfEMG abnormalities were mild in Russell's viper bit patients. All patients with neurotoxicity received the first dose of antivenom at a median of 3.75 hours before the bite. On admission, 52 patients had no clinically detectable paralysis but received antivenom due to the VICC early, yet 31 of them developed paralysis within eight hours of receiving antivenom despite the antivenom having promptly cleared the free venom from the blood circulation. This showed that the paralysis in Russell's viper envenoming is mild yet refractory to antivenom.

Antivenom should be given early for better effectiveness

The clinical studies showed antivenom was effective for VICC in Russell's viper envenoming. Identifying patients with VICC early and providing antivenom without delay would prevent any further consumption of clotting factors in the circulation, allowing the liver to replace the consumed clotting factors as early as possible. Further, studies on Indian krait and Russell's viper neurotoxicity suggested that antivenom's role in neurotoxic envenoming is primarily at the blood circulation level, and antivenom is unlikely to deliver any effect on the already initiated neuro-muscular damage. The above hypothesis was further supported by the experimental studies where the antivenom could bind with pre-synaptic β-neurotoxins of venom and prevent neurotoxicity when antivenom molecules bind with neurotoxins before they reach neuromuscular junctions, but antivenom cannot prevent the neurotoxicity if the neurotoxins were allowed to act on neuromuscular junctions first and then added the antivenom a few minutes later[39].

Based on that, it could be assumed that the motor-nerve terminal damage in Indian krait and Russell's viper bite patients had already begun before the antivenom therapy. Once the pre-synaptic neurotoxins enter the motor nerve terminal and initiate irreversible structural damage, it would be impossible for the antivenom to reverse the nerve terminal damage, which has already happened. Therefore, early antivenom therapy could benefit these patients, preventing neuromuscular junctions from getting further affected.

Using in-vitro experimentations on chick-biventer nerve-muscle preparations, the neutralisation of myotoxic activity induced by Russell's viper[32,40] and common cobra[30] venoms from Sri Lanka by Indian polyvalent antivenom was investigated. These studies clearly demonstrated that the Indian antivenoms have antibodies to effectively bind and neutralise the myotoxins in Russell's viper and common cobra venoms. However, the antivenom failed to reverse already initiated myotoxic activity in-vitro. Similar to pre-synaptic neurotoxicity, myotoxicity is also impossible to be reversed by antivenoms. Therefore, the realistic aim of antivenom therapy for myotoxicity induced by these venoms is to maximise the trapping of myotoxins in circulation by antibodies before they reach their target sites in muscles. Therefore, early antivenom therapy could benefit these patients, preventing further muscle damage.

Unreliable bedside clotting tests delay the antivenom therapy and waste valuable time: a neglected issue in Sri Lanka

In Anuradhapura Snakebite Cohort, 86% of patients directly present to the hospital without any intentional delay in seeking treatment[41]. The median time from the bite to admission for patients presenting directly to Teaching Hospital Anuradhapura was 60 min, while those first presenting to a local hospital presented a median of 40 min after the bite. These patients ultimately received their first dose of antivenom at the Teaching Hospital Anuradhapura, a median of 230 min after the snakebite. Despite trusting the healthcare system and admitting to the hospital as early as possible, snakebite patients had to wait several times longer to receive their first antivenom dose. The reason for this delay was the delay in diagnosing systemic envenoming. Because by the time the patients presented to the hospital, the clinical features of systemic envenoming had not been developed in a detectable manner. The antivenom is given only if the systemic envenoming is certain because of the poor safety profile of Indian polyvalent antivenom concerning the acute adverse reaction. This forces the healthcare staff to observe and wait for the development of clinical features of systemic envenoming.

A recent study investigated the utility of bedside clotting tests in diagnosing VICC in Anuradhapura and found that 159/272 (58%) patients with Russell's viper and Hump-nosed viper envenoming had VICC on admission. The study showed that 20 min whole blood clotting test (WBCT20) has a sensitivity of 31% in detecting VICC with INR>1.5 in these patients, and a sensitivity of 57% in detecting VICC with INR>3[42]. This shows that WBCT20 misses detecting 70% of patients with VICC on admission, which makes the treating staff wait for the VICC to become severe enough for WBCT20 to detect, wasting valuable time. Therefore, better tests are needed to detect envenoming early. In this regard, a recent study showed biomarkers such as secretary phospholipase A2 (sPLA2) to be more sensitive in predicting VICC and systemic envenoming, hence its potential to be developed as a rapid test[43]. However, until such a test is available, INR should be used whenever available to diagnose and monitor VICC[44].

Conclusion

Clinically focused laboratory experimentation and clinical studies, supported by improved observation tools, helped understand the efficacy, effectiveness, and limitations of Indian polyvalent antivenom in treating snake envenoming in Sri Lanka. Indian polyvalent antivenoms are largely efficacious for clinically important envenoming toxic effects of Russell's viper, Saw-scaled viper, common cobra and Indian krait venoms from Sri Lanka. The clinical effectiveness of the Indian polyvalent antivenom could largely be dependent on early diagnosis of envenoming and its administration without delay. However, significant challenges, such as frequent, severe acute adverse reactions to Indian polyvalent antivenom and poor detection tests for envenoming, are the burning issues that need prompt attention.

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